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Steryl epoxide, secobutanolide and butanolides from the stem wood of *Machilus zuihoensis*

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Abstract

A steryl epoxide, machillene (**1**), a secobutanolide, secomahubanolide (**2**), and two butanolides, zuihoenalide (**3**), and 3-(1-methoxyoctadecyl)-5-methylene-5*H*-furan-2-one (**4**), together with 12 known compounds, were isolated from stem wood of *Machilus zuihoensis*. Their structures were determined by means of spectroscopic analyses. Machillene (**1**) showed cytotoxic activity against NUGC-3 and HONE-1 cancer cell lines in vitro.

Keywords: *Machilus zuihoensis*; Lauraceae; Stem wood; Steryl epoxide; Machillene; Secobutanolide; Secomahubanolide; Butanolides; Zuihoenalide; 3-(1-Methoxy-octadecyl)-5-methylene-5*H*-furan-2-one; Cytotoxicity

1. Introduction

Machilus zuihoensis Hayata (Lauraceae), an endemic species in Taiwan, is a medium-sized evergreen tree found widely throughout the island, from the low lands up to an altitude of 1500 m (Liao, 1996). Its bark is one of the materials used in making incense sticks. Earlier investigations have established the presence of the two alkaloids, *L*-(−)-*N*-norarmepavine and (±)-*N*-norarmepavine, in the stem (Tomita et al., 1965) of this plant, and four neolignans in the bark (Lee, 1981). The stem wood has shown significant cytotoxicity on high-throughput screening against NUGC-3 and HONE-1 cancer cell lines in vitro. The cytotoxic constituents methyl(2*E*)-2-(1-hydroxy-2-oxopropyl)eicos-2-enoate and β-bisabolol, four new butanolides and one new dihydroxybisabolol were reported in our previous study (Cheng et al., 2002). Continuing examination of the stem wood has now resulted in the characterization of four new compounds: machillene (**1**), secomahubanolide (**2**), zuihoenalide (**3**), and 3-(1-methoxyoctadecyl)-5-methylene-5*H*-furan-2-one (**4**), together with 12 known compounds. The isolation, structural elucidation of these new compounds and cytotoxicities of some isolates are described herein.

2. Results and discussion

Machillene (**1**) was obtained as a colourless oil with the molecular formula $C_{29}H_{36}O_2$, as determined by EI–MS ($[M]^+$, *m/z* 416) and HR-EI–MS. The UV spectrum showed absorption maxima at 259 nm, indicating the presence of a benzenoid moiety (Scott, 1964). IR absorptions were observed at 1559 cm^{-1} for the benzene ring. The ^1H NMR spectrum of **1** showed two AA′XX′ signals at δ 7.09 (4H, m) and δ 7.13 (4H, m) suggesting

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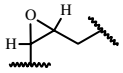
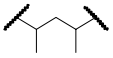
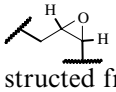
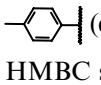
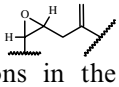
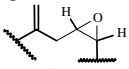
two 1,4-disubstituted benzene rings in **1**. In addition, the ^{1}H NMR and HMQC spectra revealed the presence of two methyls attached to two aromatic rings [δ 2.32 (6H, *s*, ArMe)], respectively; four oxymethines on two epoxy rings [δ 2.72 (1H, *td*, J = 6.0, 2.4 Hz, H-2), 2.83 (1H, *td*, J = 6.0, 2.4 Hz, H-10), 2.90 (1H, d, J = 2.4 Hz, H-1), 3.09 (1H, *d*, J = 2.4 Hz, H-11)]; two methyl groups [δ 1.30 (6H, *d*, J = 7.0 Hz, H-13, 14)]; two exo-methylene protons [δ 4.85 (1H, *d*, J = 1.6 Hz, H_a-12), 4.92(1H, *br s*, H_b-12), 4.95 (1H, *d*, J = 1.6 Hz, H_a-15), 5.07 (1H, *d*, J = 1.6 Hz, H_b-15); three methylene protons [δ 1.65, 2.00 (each 2H, *m*, H-9, 3), 1.81 (2H, *m*, H-6)]; and two methine protons [δ 2.83–2.95 (2H, *m*, H-5, 7)]. The COSY spectrum established the presence of the partial structures  (**a**),  (**b**),  (**c**), and  (**d**). The skeleton of **1** was constructed from the HMBC spectrum (Fig. 2). The ^2J and ^3J correlations of the signal at δ 1.65, 2.00 (H-3) with the carbon resonance at δ 143.2 (C-4) and δ 113.1 (C-12), and of the signal at δ 1.65, 2.00 (H-9) with the resonances at δ 143.6 (C-8) and δ 113.6 (C-15), helped to establish the connections of fragments **a** and **c** with the exocyclic olefinic protons at C-4 and C-8, respectively. Thus, fragments **a** and **c** can be redrawn as  (**e**) and  (**f**). Other ^3J correlations in the HMBC spectrum of **1** were observed between δ 1.65, 2.00 (H-3) and δ 36.9 (C-5), and δ 1.65, 2.00 (H-9) and δ 37.4 (C-7), suggesting that fragments **e** and **f** were linked at C-5 and C-7. Other significant correlations in the HMBC of **1** were observed from δ 2.90 (H-1) to δ 126.6 (C-2′, 6′), and δ 3.09 (H-11) to δ 126.6 (C-2″, 6″), suggesting fragments **e/d** and **f/d** were linked at C-1 and C-11. The structure of **1** could thereby be established. The absence of NOESY contacts between H-1/H-2 and H-10/H-11, and the value of the coupling constant (Stierle et al., 1998) (J = 2.4 Hz), indicate that H-1/H-2 and H-10/H-11 are *trans*-form, respectively. Thus, the structure of machillene was elucidated as **1**, which was further confirmed by ^{13}C NMR, DEPT, COSY, NOESY (Fig. 1), HMQC,and HMBC (Fig. 2) analyses.

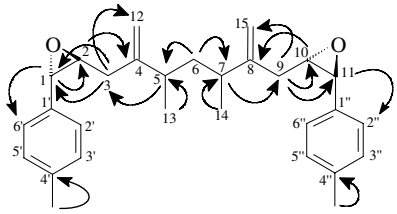
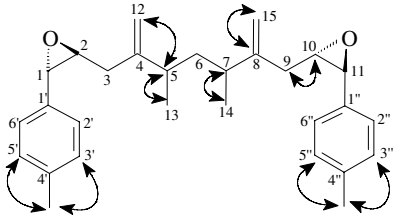


Fig. 2. Key HMBC correlations of **1**.

Secomahubanolide (**2**) was isolated as a colourless oil. Its molecular formula, $C_{24}H_{44}O_4$, was established by FAB-MS ([M + H]$^+$, *m/z* 397) and HR-FAB-MS. The UV absorption at 215 nm was similar to that of secolincomolide A (Tsai et al., 2002), indicating the presence of a secobutanolide skeleton (Tsai et al., 2002). The IR spectrum of **2** showed characteristic absorption bands due to the presence of hydroxyl (3440 cm^{-1}), ester (1730 cm^{-1}), and ketone (1710 cm^{-1}) groups. The ^{1}H NMR spectrum of **2** was similar to that of secolincomolide A (Tsai et al., 2002), except that the *Z*-form geometry of the trisubstituted double bond [δ 6.34 (1H, *t*, J = 7.6 Hz, H-3)] in **2** was in place of the *E*-form geometry [δ 7.08 (1H, t, J = 7.6 Hz, H-3)] in secolincomolide A (Tsai et al., 2002). Compound **2** showed six more methylene units [δ 1.27 (28H, *br s,* H-6–19)] than secolincomolide A [δ 1.27 (16H, *br s*, H-6–H-13)] in the side-chain. An acetyl and one methoxyl groups were observed at δ 2.19 (3H, s, H-3′), and 3.74 (3H, *s*, OMe-1), respectively. Compound **2** showed the laevorotatory optical activity [$[\alpha]_D^{25}$: −37.2° (*c* 0.029, $CHCl_3$)] indicated the 1′*R* configuration like secolincomolide A [$[\alpha]_D^{25}$: −11.3° (*c* 0.098, $CHCl_3$)] (Tsai et al., 2002), which was contrary to secoisolancifolide [$[\alpha]_D^{25}$: +102.7° (*c* 0.49, $CHCl_3$)] (Tanaka et al., 1989). From the above data, compound **2** was elucidated as (2*Z*)-2-[(1*R*)-1-hydroxy-2-oxo-propyl]-icos-2-enoic acid methyl ester, and its structure was represented as **2**, as was further confirmed by COSY and ^{13}C NMR experiments.

Zuihoenalide (**3**) was isolated as a colourless oil. The molecular formula was determined to be $C_{23}H_{40}O_3$ by FAB-MS ([M + H]$^+$, *m/z* 365) and HR-FAB-MS. The UV absorptions at 227 nm of **3** was similar to that of isolincomolide D (Cheng et al., 2001), indicating the



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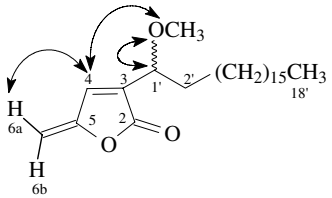

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Fig. 1. Key NOESY correlations of **1** and **4**.

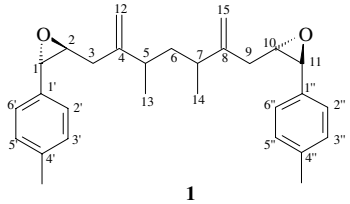
presence of β-hydroxy-γ-methylene-α,β′-unsaturated-γ-lactone ring (Cheng et al., 2001). The IR spectrum showed absorption bands for a hydroxyl group at 3450 cm^{-1} and an α,β-unsaturated-γ-lactone ring at 1766 and 1682 cm^{-1}. The ^1H NMR spectra of **3** was similar to that of isolincomolide D (Cheng et al., 2001), indicating that **3** has the same β-hydroxy-γ-methylene-α,β′-unsaturated-γ-lactone skeleton and the same *Z* geometry of the trisubstituted double bond [δ 6.69(1H, *td*, *J* = 8.0, 2.0 Hz, H-1′)]. The presence of a broad singlet δ 1.26 (28H, *br s*, H-4′–17′) was attributed to protons in a long methylene chain in **3**. The exocyclic olefinic protons appeared at δ 4.67, 4.89 (each 1H, *dd*, *J* = 2.8, 1.5 Hz, H-6a, b) and one hydroxymethine proton located at δ 5.11 (1H, *br s*, H-4). Compound **3** showed an $[\alpha]_D^{25}$ value of –50.2° (*c* 0.015, $CHCl_3$),indicating the *S* configuration at C-4 like 2-octadecylidene-3-hydroxy-4-methylenebutanolide [$[\alpha]_D^{25}$: –38.5° (*c* 0.40, $CHCl_3$)] (Martinez et al., 1981). Thus, the structure of zuihoenalide was represented as **3**, and elucidated as (4*S*,3*Z*)-4-hydroxy-5-methylene-3-octadecylidene-dihydro-furan-2-one, which was further confirmed by COSY and NOESY experiments.

3-(1-Methoxyoctadecyl)-5-methylene-5*H*-furan-2-one (**4**) was isolated as colourless oil. The molecular formula was determined to be $C_{24}H_{42}O_3$ by EI-MS ($[M]^+$, *m*/*z* 378) and HR-EI-MS. The presence of an α,β-unsaturated-γ-lactone moiety was apparent from UV absorption at 264 nm (Tanaka et al., 1989). The IR spectra showed an α,β-unsaturated-γ-lactone ring at 1780 and 1680 cm^{-1}. In the ^1H NMR spectrum, the signals of an olefinic proton at δ 7.21 (1H, *br s*, H-4), exomethylene proton at δ 4.87, 5.20 (each 1H, d, *J* = 1.6 Hz, H-6a, 6b), an oxymethine proton at δ 4.12 (1H, *dd*, *J* = 6.0, 4.4 Hz) which was similar with those of the known butanolide, 2-(1-methoxy-11-dodecenyl)-penta-2,4-dien-4-olide (Kwon et al., 2000), except that a 1-methoxyoctadecyl group [δ 0.88 (3H, *t*, *J* = 6.8 Hz, H-18′), 1.25 (28H, *br s*, H-4′–17′), 1.61–1.80 (4H, *m*, H-2′, 3′), 3.35 (3H, *s*, OCH_3-1′)] in **4** was in place of a 1-methoxy-11-dodecenyl group [δ 1.26 (10H, *br s*, H-4′-8′), 1.35 (4H, *m*, H-3′, 9′), 1.64–1.74 (2H, *m*, H-2′), 2.03 (2H, *m*, H-10′), 3.34 (3H, *s*, OCH_3-1′), 4.93 (1H, *dm*, *J* = 10.5 Hz, H-12′a), 4.99 (1H, *dtd*, *J* = 17.0, 2.3, 1.7 Hz, H-12′b), 5.81 (1H, *ddt*, *J* = 17.0.10.5, 6.5 Hz, H-11′)] in the C-3 position of the furan ring of the latter compound. Thus, the structure of **4** was elucidated as 3-(1-methoxyoctadecyl)-5-methylene-5*H*-furan-2-one, which was further confirmed by COSY and NOESY (Fig. 1) experiments. Compound **4** has laevorotatory optical activity, but the configuration at C-1′ remains undefined.

The known compounds included four benzenoids: methyl paraben (Cheng et al., 2003), sinapic aldehyde (Cheng et al., 2003), kaoburaide (Kanchanapoom et al., 1994), and α-tocopherol (Kuo et al., 2002); one amide: *N*-*trans*-feruloyltyramine (Chang et al., 2000a); four steroids: β-sitosterol (Chang et al., 2000b), β-sitosteryl glucoside (Chang et al., 2000b), β-sitostenone (Chang et al., 2000b), and stigmastan-3β,5α,6β-triol (Meyer et al., 1998); one organic acid: (+)-abscisic acid (Hampson et al., 1992); one triterpenoid: friedelin (Wang et al., 2002); and one sugar: *n*-butyl-β-D-fructopyranoside (Zhang et al., 1996). These compounds were identified by comparisons of their spectral data (UV, IR, ^1H NMR, MS) with the data from the corresponding values in the literature, or with authentic samples.

The cytotoxic activity of the following three compounds, machillene (**1**), zuihoenalide (**3**), and kaoburaide, and two compounds, methyl (2*E*)-2-(1-hydroxy-2-oxopropyl)eicos-2-enoate and β-bisabolol in previous report (Cheng et al., 2002) were tested in vitro against NUGC-3 and HONE-1 cell lines and **1**, (2*E*)-2-(1-hydroxy-2-oxopropyl)eicos-2-enoate and β-bisabolol showed significant cytotoxicity (0%, 5%; 0%, 0%; 1%, 0%), as interpreted by percentage of cell survival at concentrations of 20 μg/mL. However, the other two compounds did not display any cytotoxicity against these two cell lines. The known *N*-*trans*-feruloyltyramine with cytotoxicity against P-388 and HL-60 cancer cell lines was already reported (Wu et al., 1996).

In conclusion, four new optically active compounds (**1**–**4**) were isolated as minor constituents in further study from the stem wood of this plant. The following known compounds, kaoburaide, stigmastan-3β,5α,6β-triol, (+)-abscisic acid, and *n*-butyl-β-D-fructopyranoside were firstly isolated in *Machilus* species.



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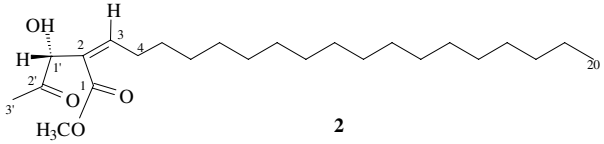

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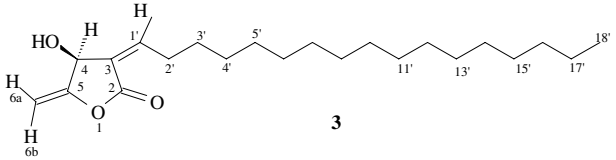

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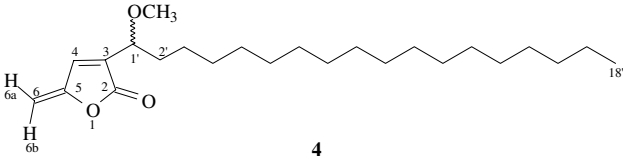

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3. Experimental

3.1. General

Mps are uncorr. ^{1}H NMR (600, 500, 400 and 200 MHz) and ^{13}C NMR (150, 125, 100 and 50 MHz) were taken in $CDCl_3$. Chemical shifts are given in δ with TMS as int. standard. EI-mass spectra were recorded on a VG Biotech Quattro 5022 spectrometer. HR-mass spectra were recorded on a JEOL JMX-HX 110 spectrometer. Optical rotations were measured using a Jasco P-1020 polarimeter in $CHCl_3$. All melting points were determined on a Yanaco micro-melting point apparatus and were uncorrected. IR spectra were taken on a Genesis II FTIR spectrophotometer. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer in EtOH. Silica gel (60–230, 230–400 mesh) (Merck) was used for CC and silica gel 60F-254 (Merck) for prep. TLC.

3.2. Plant material

The stem wood of *M. zuihoensis* was collected at Mutan, Pingtung County, Taiwan, in January 2000. A voucher specimen (No. Chen 2280) was deposited in the Herbarium of the School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan, R.O.C.

3.3. Extraction and isolation

The stem wood (23.9 kg) was chipped, extracted with cold MeOH and concentrated under reduced pressure. The MeOH extract (1000 g) was partitioned between *n*-hexane and H_2O (1:1) (1000 mL × 5) followed by EtOAc (1000 mL × 5) to obtain an *n*-hexane soluble layer (fraction A, 640 g), an EtOAc soluble layer (fraction B, 13.4 g) and an H_2O soluble layer (fraction C, 160 g) after evaporating the solvents.

Part of fraction A (80 g) was subjected to Si gel chromatography by eluting with *n*-hexane–EtOAc (50:1), then enriched with EtOAc to furnish 15 fractions (A-1–A-15). Fraction A-1 (1.0 g) was resubjected to Si gel chromatography, eluting with *n*-hexane–EtOAc (10:1) and enriched gradually with EtOAc to obtain 15 fractions (A1-1–A1-15). Fraction A1-7 (20 mg) was purified by preparative TLC (*n*-hexane–EtOAc, 10:1) to yield friedelin (7.1 mg). Fractions A-4 (185 mg) and A-5 (3910 mg) were resubjected to Si gel CC and purified by preparative TLC to yield secomahubanolide (**2**) (1.1 mg) and 3-(1-methoxyoctadecyl)-5-methylene-5*H*-furan-2-one (**4**) (3.2 mg). Fraction A-7 (2031 mg) was applied to a Si gel column, eluting with a *n*-hexane–Me_2CO gradient, to obtain three fractions (A7-1–A7-3). Fraction A7-2 (24.2 g) was purified by preparative TLC (*n*-hexane–EtOAc, 15:1) to yield β-sitostenone (1.9 mg) and α-tocopherol (12.5 mg).

Fraction B (13.4 g) was subjected to Si gel chromatography by eluting with $CHCl_3$–MeOH (50:1) and enriched with MeOH to afford eight fractions (B-1–B-8). Fraction B-1 (1.34 g) was also applied to a Si gel column, eluting with *n*-hexane–EtOAc (10:1) and enriched gradually with EtOAc to obtain 10 fractions (B-1-1–B-1-10). Fraction B-1-3 (124 mg) was purified by preparative TLC (*n*-hexane–EtOAc, 15:1) to give methyl paraben (2.4 mg), sinapic aldehyde (2.6 mg), and β-sitosterol (12.1 mg). Fraction B-3 (5.3 g) was subjected to Si gel chromatography, eluting with *n*-hexane–EtOAc (20:1) and enriched gradually with EtOAc to obtain five fractions (B-3-1–B-3-5). Fraction B-3-3 (2.31 g) was further separated by silica gel columns and preparative TLC (CH_2Cl_2–EtOAc, 8:1) to give β-sitosteryl glucoside (3.9 mg) and *N-trans*-feruloyltyramine (5.9 mg). Fraction B-7 (1.91 g) was subjected to Si gel chromatography, eluting with $CHCl_3$–MeOH (5:1) and enriched gradually with MeOH to obtain 10 fractions (B-7-1–B-7-10). Fraction B7-4 (265 mg) was purified by preparative TLC ($CHCl_3$–MeOH, 18:1) to give zuihoenalide (**3**) (0.7 mg), machillene (**1**) (3.9 mg), and kaoburaide (10.1 mg). Fraction B-8 (1.21 g) was subjected to Si gel chromatography, eluting with $CHCl_3$–MeOH (2:1) and enriched gradually with MeOH to obtain five fractions (B-8-1–B-8-5). Fraction B-8-3 (500 mg) was purified by preparative TLC ($CHCl_3$–MeOH, 25:1) to give stigmastan-3β,5α,6β-triol (1.6 mg).

Part (20 g) of Fraction C (160 g) was applied to Diaion HP-20 column, eluting with H_2O and gradually decreasing the polarity with MeOH, to yield 10 fractions (C-1–C10). Fraction C-2 (250.8 mg) was subjected to Si gel column chromatography, eluting with CH_2Cl_2–MeOH (5:1) to obtain 10 fractions (C-2-1–C-2-10). Fraction C-2-5 (33 mg) was purified by preparative TLC (CH_2Cl_2–MeOH, 5:1) to afford (+)-abscisic acid (1.8 mg) and *n*-butyl-β-D-fructopyranoside (7.9 mg).

3.3.1. Machillene (*1*)

Colourless oil; $[\alpha]_D^{25}$: +22.2° (c = 0.094, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 259 (4.14); IR (Neat) ν_{max} 1559 (benzene ring) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.30 (6H, *d*, J = 7.0 Hz, H-13, 14), 1.65 (2H, *m*, H-3, 9), 1.81 (2H, *m*, H-6), 2.00 (2H, *m*, H-3, 9), 2.32 (6H, *s*, ArMe), 2.72 (1H, *td*, J = 6.9, 2.4 Hz, H-2), 2.83–2.95 (2H, *m*, H-5, 7), 2.83 (1H, *td*, J = 6.0, 2.4 Hz, H-10), 2.90 (1H, *d*, J = 2.4 Hz, H-1), 3.09 (1H, *d*, J = 2.4 Hz, H-11), 4.85 (1H, *d*, J = 1.6 Hz, H_a-12), 4.92 (1H, *br s*, H_b-12), 4.95 (1H, *d*, J = 1.6 Hz, H_a-15), 5.07 (1H, *d*, J = 1.6 Hz, H_b-15), 7.09 (4H, *m*, H-3′, 5′, 3″, 5″), 7.13 (4H, m, H-2′, 6′, 2″, 6″). ^{13}C NMR (100 MHz): δ 21.0 (ArMe), 21.7 (C-13), 22.7 (C-14), 36.9 (C-5 or C-7), 37.4 (C-7 or C-5), 40.7 (C-9), 41.1 (C-3, 6), 57.1 (C-2), 57.6 (C-10), 60.9 (C-1), 61.2 (C-11), 113.1 (C-12 or C-15), 113.6 (C-15 or C-12), 126.6 (C-2′, 6′, 2″, 6″), 129.2 (C-3′, 5′, 3″, 5″), 135.2 (C-1′, 1″), 141.5 (C-4″), 143.2

(C-4″), 143.6 (C-8); EIMS *m/z*: 416 $[M]^+$; HREIMS *m/z*: 416.2714 (calcd for $C_{29}H_{36}O_2$: 416.2718).

*3.3.2. Secomahubanolide (**2**)*

Colourless oil; $[\alpha]_D^{25}$: −11.16° (*c* 0.029, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 215 (3.71); IR (Neat) ν_{max} 3440 (OH), 1730 (ester), 1710 (ketone) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (3H, *t*, *J* = 6.8 Hz, H-20), 1.27 (28H, *br s*, H-6–19), 1.47 (2H, *m*, H-5), 2.19 (3H, *s*, H-3′), 2.54 (2H, *q*, *J* = 7.6 Hz, H-4), 3.74 (3H, *s*, OMe-1), 4.04 (1H, *br d*, *J* = 3.2 Hz, OH-1′, D_2O exchangeable), 4.53 (1H, *br d*, *J* = 3.2 Hz, H-1′), 6.34 (3H, *t*, *J* = 7.6 Hz, H-3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1 (C-20), 24.7 (C-3′), 26.9 (C-5), 27.2 (C-4), 29.0–30.0 (C-6–19), 51.6 (OMe-1), 80.4 (C-1′), 129.9 (C-2), 149.4 (C-3), 166.5 (C-1), 206.3 (C-2′); FABMS *m/z*: 397 $[M + H]^+$; HRFABMS *m/z*: 397.3240 (calcd for $C_{24}H_{45}O_4$: 397.3250).

*3.3.3. Zuihoenalide (**3**)*

Colourless oil; $[\alpha]_D^{25}$: −50.2° (*c* 0.015, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 227 (4.17); IR (Neat) ν_{max} 3450 (OH), 1766, 1682 (α,β-unsaturated-γ-lactone ring) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (3H, *t*, *J* = 6.8 Hz, H-18′), 1.26 (28H, *br s*, H-4′–17′), 1.50 (2H, *m*, H-3′), 2.76 (2H, *m*, H-2′), 4.67, 4.89 (each 1H, *dd*, *J* = 2.8, 1.5 Hz, H-6a, b), 5.11 (1H, *br s*, H-4), 6.69 (1H, *td*, *J* = 8.0, 2.0 Hz, H-1′); FABMS *m/z*: 365 $[M + H]^+$; HRFABMS *m/z*: 365.2985 (calcd for $C_{23}H_{41}O_3$: 365.2990).

*3.3.4. 3-(1-Methoxy-octadecyl)-5-methylene-5H-furan-2-one (**4**)*

Colourless oil; $[\alpha]_D^{25}$ −13.7° (*c* 0.0091, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 264 (3.72) nm; IR (Neat) ν_{max} 3450, 1780, 1680 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 0.88 (3H, t, *J* = 6.8 Hz, H-18′), 1.25 (28H, *br s*, H-4′–17′), 1.61–1.80 (4H, *m*, H-2′, 3′), 3.35 (3H, *s*, OMe-1′), 4.12 (1H, *dd*, *J* = 6.0, 4.4 Hz, H-1′), 4.87, 5.20 (each 1H, *d*, *J* = 1.6 Hz, H-6a, b), 7.21 (1H, *br s*, H-4); EIMS *m/z*: 378 $[M]^+$; HREIMS *m/z*: 378.3133 (calcd for $C_{24}H_{42}O_3$: 378.3133).

3.4. Cytotoxicity assay

Human HONE-1 cells (nasopharyngeal carcinoma, from FIRDI, Taiwan), and NUGC-3 cells (gastric adenocarcinoma) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and nonessential amino acid (Life Technologies, Inc.), and maintained at 37 °C in a humidified incubator with an atmosphere of 5% CO_2.

Human cancer cells were seeded in 96-well microtiter plates at a density of 6000/well in 100 μl culture medium. After an overnight adaptation period, 50 μg/ml (final concentration) of test compounds in serum-free medium were added to individual wells. Cells were treated with test compounds for 3 days. Cell viability was determined by the 5-(3-carboxymethoxyphenyl)-2-(4,5-dimethylthiazoyl)-3-(4-sulfophenyl) tetrazolium salt (MTS) reduction assay. Actinomycin D 5 μM (final concentration) and DMSO 0.3% (final concentration) were used as positive and vehicle controls. Results were expressed as a percentage of DMSO control.

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